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INVENTOR(S)/APPLICANT(S)			
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TITLE OF INVENTION (280 characters max)
TREATMENT AND PREVENTION OF RENAL DYSFUNCTION

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☒ Applicant is a Small Entity.

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PROVISIONAL APPLICATION FILING ONLY

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
APPLICATION FOR UNITED STATES LETTERS PATENT

PROVISIONAL APPLICATION

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TITLE: TREATMENT AND PREVENTION OF RENAL DYSFUNCTION

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TREATMENT AND PREVENTION OF RENAL DYSFUNCTION

FIELD OF THE INVENTION

The present invention generally relates to the field of nephrology and more specifically relates to methods for treating and preventing renal dysfunction, including, but not limited to, acute renal failure, transplant-associated acute renal failure, and chronic renal failure.

BACKGROUND OF THE INVENTION

Renal dysfunction, including acute renal failure, transplant-associated acute renal failure, and chronic renal failure, remains a major, unresolved medical problem. For example, despite the substantial progress that has been made in the treatment of various renal diseases, patient mortality associated with acute renal failure (ARF) has remained ≥ 50%. In patients with end stage renal disease (ESRD) and other types of chronic renal failure (CRF), the annual rise in the patient population is about 9%.

ARF is defined as an acute deterioration in renal function within hours or days, resulting in the accumulation of toxic metabolites that are normally eliminated by the kidney. The most common cause of ARF is ischemic injury of renal tubular and postglomerular vascular endothelial cells. The principal etiologies for this ischemic form of ARF include intravascular volume contraction, resulting from bleeding, thrombotic events, shock, sepsis, major cardiovascular surgery, arterial stenoses, and others. Nephrotoxic forms of ARF are caused by radiocontrast agents, and frequently used medications such as chemotherapeutic agents, antibiotics and cyclosporine. Patients most at risk for ARF include diabetics, underlying kidney disease, the elderly, underlying vascular disease, liver and cardiac diseases, and cancer.

Both ischemic and nephrototoxic forms of ARF result in death of tubular cells. Sublethally injured tubular cells dedifferentiate, lose their polarity and express vimentin, a mesenchymal cell marker, and Pax-2, a transcription factor that is normally only expressed in the process of mesenchymal-epithelial transdifferentiation in the embryonic kidney.

The kidney, even after severe acute insults, has the remarkable capacity of self-regeneration and consequent re-establishment of nearly normal function. It is thought that the regeneration of injured nephron segments is the result of migration, proliferation and redifferentation of surviving tubular and endothelial cells. However, the self-regeneration capacity of the surviving tubular and endothelial cells is exceeded in ARF. A need exists to enhance the kidney's self-regeneration capacity after severe injury.

Another acute form of renal failure, transplant-associated acute renal failure (TA-ARF), often develops due to kidney transplantation, mainly in patients receiving transplants from cadaveric donors, although TA-ARF may also occur in patients receiving a living related donor kidney. Cadaveric donors represent about 50% of the kidney transplants currently performed. The kidney recipients regularly develop early graft dysfunction, resulting in loss of kidney function and requiring treatment with hemodialysis until graft function recovers. The risk of TA-ARF is increased with elderly donors, marginal graft quality, and an extended period of time between harvest of the donor kidney from a cadaveric donor and it implantation into the recipient, known as "cold ischemia time". Early graft dysfunction due to TA-ARF has serious long-term consequences, including accelerated graft loss due to progressive, irreversible loss in kidney function that is initiated by TA-ARF and an increased incidence of acute rejection episodes leading to premature loss of the kidney transplant. Therefore, a need exists to provide a treatment or prevention of early graft dysfunction due to TA-ARF.

Chronic renal failure (CRF) is the progressive loss of nephrons and subsequent loss of renal function. The progressive loss of nephrons appear to result from a self-perpetuating fibrotic and sclerosing process most prominently manifested in the renal interstitium. Glomerular, vascular and inflammatory injuries to the kidney appear in parallel to the nephritic injuries resulting in the eventual loss of tubular cells.

The loss of tubular cells and renal function, whether occurring in acute or chronic renal failure, is a serious medical condition that will be ameliorated by the present invention. Any slowing, arrest, or reversal of the decline in

renal function provided by the present invention will be enormously beneficial to the affected patients with ARF, TA-ARF, CRF, or any kidney-associated dysfunction.

DETAILED DESCRIPTION OF THE INVENTION

The present invention will utilize pluripotent stem cells for the repair of critically damaged kidney tissues. The pluripotent stem cells may also be utilized as a preventative measure, for example in patients at risk for developing ARF or TA-ARF or CRF. The present invention may also utilize endogenous stem cells that have been mobilized with colony stimulating factors, cytokines, or stem cell factors to provide a source of the pluripotent stem cells.

Stem cells may be utilized to repopulate dysfunctional kidneys because of the "plasticity" of stem cell populations. The term "plasticity" refers to the phenotypically broad differentiation potential of cells that originate from a defined stem cell population. Accordingly, stem cell plasticity can include differentiation of stem cells derived from one organ into cell types of another organ. "Transdifferentiation" refers in the strictest sense to the ability of a fully differentiated cell, derived from one germinal cell layer, to differentiate into a cell type that is derived from another germinal cell layer.

It was assumed, until recently, that stem cells during organogenesis lose gradually their pluripotency and thus their differentiation potential and the differentiation potential of somatic stem cells was restricted to cell types of the organ from which stem cells originated. In addition, this differentiation process was thought to be unidirectional and irreversible. However, recent studies have shown that somatic stem cells maintain their differentiation potential. For example, it was demonstrated that hematopoietic stem cells are able to transdifferentiate into muscle, neurons, liver, myocardial cells, and kidney. However, transdifferentiation processes under normal, steady state conditions are extremely rare. It is furthermore possible that as yet undefined signals that originate from injured and not from intact tissue act as transdifferentiation signals.

The present invention will utilize pluripotent stem cell populations to treat or prevent renal dysfunction. Non-transformed, adult stem cells will be used to repopulate kidney cells to support and augment the repair processes in patients with renal dysfunction either by integration of stem cells into the tubular epithelium and/or endothelium or indirectly by intrarenal secretion of renotropic survival factors. The pluripotent stem cell populations used to repopulate the dysfunctional kidney may be derived from hematopoietic, mesenchymal, kidney, liver, muscle, or fat stem cells. Of course, other cells and organs may provide a source of stem cells to repopulate dysfunctional kidneys. As used herein, "adult" stem cells refers to stem cells that are not embryonic in origin. The term "non-transformed" as used herein refers to stem cells that have not been genetically modified with exogenous DNA or RNA.

In addition to the pluripotent stem cell populations derived from hematopoietic, mesenchymal, kidney, liver, muscle, or fat stem cells, endogenous stem cell populations may be mobilized directly from the resident organs to repopulate the damaged kidney using stem cell mobilization factors.

In one embodiment of the present invention, the pluripotent stem cell population is derived from hematopoietic stem cells. The hematopoietic stem cells are derived from the bone marrow or peripheral blood, preferably the bone marrow. The hematopoietic stem cells are isolated from a donor or the patient themselves by techniques commonly known in the art. The hematopoietic stem cell population may be enriched for the pluripotent hematopoietic stem cells using fluorescence activated cell sorting (FACS). The pluripotent hematopoietic stem cells may be enriched by FACS by selecting for "c-kit" positive, "sca-1" positive and "lin negative" cells. "c-kit' and "sca-1" cells are known to one of skill in the art as being receptors known to be on the surface of stem cells. A "lin negative" cell is known to one of skill in the art as being a cell that does not express antigens characteristic of specific cell lineages and thus is more primordial. Any method know to one of skill in the art may be used to enrich the population of pluripotent stem cells from a population of bone marrow cells.

Once the hematopoietic stem cells are isolated, the stem cells may be used to treat patients with ARF, TA-ARF, CRF, or those patients at risk for developing renal disease. In patients with ARF, the hematopoietic stem cell may be transfused intravenously. The hematopoietic stem cells will enter the damaged kidneys via the circulation, home to the site of kidney injury and cell loss, undergo local differentiation into tubular cells and thereby boost the regeneration process that will improve kidney function.

In patients at risk for developing TA-ARF or with established TA-ARF, hematopoietic stem cells from the kidney donor or from the kidney recipient may be transfused intraoperatively (intravenously) into the kidney via the circulation and improve kidney function as described for ARF patient treatment above. Alternatively, the stem cells may be transfused intrarenally. Hematopoietic stem cells may also be used to treat patients with CRF, using the methods described above for ARF treatment.

In another embodiment of the present invention, the population of pluripotent stem cells may be derived from an adherent subpopulation within the hematopoietic stem cell population. Mesenchymal stromal stem cells are derived from bone marrow cells that are placed into sterile culture *in vitro*. Hematopoietic cells will not adhere to the bottom of the culture dishes, while mesenchymal stromal stem cells will adhere to the culture dish. After discarding the non-attached cells, mesenchymal stromal stem cells will grow and expand in culture, yielding a well defined population of pluripotent stem cells. After expansion *in vitro*, the mesenchymal stromal stem cells may also be depleted for CD-45 expressing cells to remove more differentiated cells prior to administration to the patient. The mesenchymal stromal stem cells may be used to treat patients having ARF, TA-ARF, CRF or are at risk for developing renal disease. The treatment regime using mesenchymal stromal stem cells is the same as describe above using hematopoietic stem cells. Mesenchymal stromal stem cells may be derived from a compatible donor or the patient. Since the mesenchymal stromal stem cells may be expanded in vitro, the treatment regime may be repeated in order to further augment the cell replacement and repair process in the injured kidney.

The mesenchymal strómal stem cells may also be used in addition to hematopoietic stem cells to treat kidney dysfunction. The mesenchymal stromal stems cell may be transfused prior to, post, or simultaneously with the hematopoietic stem cells. Various ratios of stem cell populations may be used for treatment.

In another embodiment of the present invention, the population of pluripotent stem cells may be derived from non-hematopoietic sources such as the kidney or other tissue sources, such as the liver, muscle, or fat, or any tissue suitable as a source of pluripotent stem cells. The non-hematopoietic stem cells may be enriched in vitro and then administered to the patient as described above for the hematopoietic stem cells. The non-hematopoietic stem cells may be used to treat patients having ARF, TA-ARF, CRF or are at risk for developing renal disease.

In another embodiment of the present invention, the patient's own stem cells may be used to treat kidney dysfunction by mobilizing endogenous stem cells. The stem cells may be mobilized with granulocyte-colony stimulating factor (G-CSF), and/or stem cell factor (SCF), or any stem cell mobilization factor known to one skilled in the art. G-CSF and SCF treatment results in the mobilization and transfer of hematopoietic and non-hematopoietic stem cells into the circulating blood. Thus, the blood that perfuses the kidneys is enriched with stem cells that are immediately available to replace kidney tubular cells that are damaged by subsequent insults such as surgery, administration of kidney damaging drugs and agents.

In certain embodiments, a therapeutically effective dose of stem cells and/or a therapeutically effective dose of G-CSF or SCF are delivered to the patient. An effective dose for treatment will be determined by the body weight of the patient receiving treatment. A therapeutic dose may be one or more administrations of the therapy.

Delivery of the stem cells may be by endogenous mobilization of the stem cells, or by injection or instillation of the stem cells into the patient. Stem cells may be injected or instilled directly into the kidney or alternatively the stem cells may be injected intravenously or intraperitoneally.

EXAMPLES

Example 1

Establishment of a Mouse Model of Ischemic ARF

The kidneys of FVB mice were subjected to various ischemia times by clamping of both renal pedicles. Resulting severity of ARF and survival rates were monitored. Two models were identified to be used in further examples discussed below. A model of severe ARF may be established using 60 minutes of bilateral renal ischemia. The 60 minute bilateral renal ischemic treatment resulted in a mortality of 50% at 72 hrs post reflow and a glomerular filtration rate of < 5% of normal. Histological examination of the severe ARF model shows wide spread tubular necrosis and severe vascular congestion in the corticomedullary junction. A moderate ARF model may be established using 55 minutes of bilateral renal ischemia. The moderate ARF model exhibits a serum creatinine level of about 1.5 mg/dL and a mortality of < 10%.

Example 2

Examine the Effect of Hematopoietic Stem Cell Mobilization on the Outcome of Ischemia/Reperfusion-Induced ARF in Mice

Ischemic ARF will be induced in anesthetized, adult FVB mice by timed clamping of both renal pedicles. Renal function will be monitored by measurement of serum creatinine levels. Prior to induction of ARF, a subgroup of mice will be treated with cytoxan, followed by G-CSF until peripheral HSC numbers, assessed by colony assay, are maximally increased. At this time, ARF will be induced and outcome over time is monitored. At the end of the observation period (3-7 days post ARF), animals will be sacrificed and kidneys will be perfusion fixated for histological analysis (injury scores, inflammatory cell infiltrates). Sham operated mice and leukocytopenic mice with ARF will serve as controls for renal function and the impact of simultaneously induced granulocytosis, respectively.

Example 3

Characterize Homing Signals and Mechanisms for Leukocytes and Stem Cells in the Kidney with ARF

The kidneys and HSC/MSC from the animals studied in Example 2 will be examined for SDF-1 and CXCR4 expression using in situ hybridization and real time RT-PCR, respectively. The importance of the chemokine SDF-1 and its receptor CXCR4 in mediating chemokinesis of HSC/MSC will be further investigated in vitro using transwell migration assays and in experiments with neutralizing anti-SDF-1 antibodies.

Determinations will be made whether the injured tubular or endothelial cells in ARF expresses SDF-1, and whether mobilized stem cells express CXCR4. This determination will provide for a system for mediation of homing of CXCR4-expressing stem cells towards the sites of nephron and vascular injury. Homing efficiency of the HSC will be optimized to improve the renoprotective stem cell therapies.

Example 4

Determine the Effect of HSC Therapy on the Outcome of ARF in Mice

In order to determine whether HSC home into the kidney in ARF, and whether they transdifferentiate, integrate and act renoprotectively, genetically marked, phenotyped cells will be exogenously administered and traced in the kidney of mice with ARF. HSC will be obtained from the femurs of eGFP transgenic FVB mice that express enhanced green fluorescent protein (eGFP+ HSC). The eGFP+ HSC will be enriched by FACS sorting (c-kit, sca-1, lin negative), and administered intravenously to wild type mice with ARF as described in Example 2 above. Appropriate controls will be included. At defined time points following induction of ARF, eGFP+ HSC will be administered and kidneys from experimental and control mice will be examined in order to assess where eGFP cells are located and whether they have transdifferentiated into renal tubular or endothelial cells, respectively. Renal function and histology is examined as above and for direct tissue

evidence of transdifferentiation and integration of eGFP+ cells into tubular or vascular endothelial sites at which ARF caused cell injury and loss. The paracrine potential of HSC and MSC, see example 5, to produce, deliver and release renoprotective growth factors in situ such as HGF, EGF, IGF-I, etc. will be tested in *in vitro* studies using ELISA assays and RT-PCR.

Example 5

Determine the Effect of MSC Therapy on Outcome of ARF in Mice

MSCs originate, like the kidneys, from the mesoderm and have been shown to transdifferentiate into numerous cell types. MSC from eGFP transgenic FVB mice will be utilized. The eGFP+ MSC will be isolated from harvested bone marrow based on their characteristic and selective attachment to the culture dish. A functional MSC culture system will be established that provides for well maintained eGFP expressing MSCs at later passages. Cultured eGFP+ MSC will be administered to wild type mice with ARF, as described in Example 4 and outcome and tissue analyses will be performed as above. The results will be analyzed to determine the MSC renoprotective effects as compared to the HSC renoprotective effects. The results may suggest that co-administration of HSC and MSC may be most beneficial, since these cells depend on each other for effective hematopoiesis. The paracrine potential of MSC to produce, deliver and release renoprotective growth factors in situ including HGF, EGF, IGF-I, etc. will be tested in *in vitro* studies using ELISA assays and RT-PCR.

Example 6

Assess the Effect of MSC Therapy on the Function of Renovascular Endothelial Cells in ARF

The kidney is a highly perfused organ, receiving 20% of the cardiac output, and the complexity of the intrarenal circulation facilitates the processes of filtration and tubular transport. It is now recognized that vascular endothelial cell dysfunction and death are important determinants of loss of

renal function in ARF. The bone marrow contains endothelial precursor cells (CEP, circulating endothelial precursors), that can be mobilized into the peripheral circulation, from where they can contribute to wound healing or participate in tumor angiogenesis. Both bone marrow-derived stem cells types, HSC and MSC, are able to transdifferentiate into endothelial cells. The effect of MSC will be targeted, after transdifferentiation into endothelial cells, or c-kit+/VEGFR2+ hematopoietic cells, from eGFP transgenic mice, on the course of ARF. The cell type that will be assessed in these experiments is the postglomerular vascular endothelial cell that is injured in ARF.

MSC and c-kit+/VEGFR2+ hematopoietic cells from eGFP transgenic mice (CEP) will be subjected to various transdifferentiation protocols in vitro with the goal of obtaining endothelial cells, phenotypically confirmed by appropriate endothelial cell markers. Cells will then be administered to mice with ARF as in the preceding protocols (Examples 4 and 5) and their impact on the course of ARF will be monitored as above. Kidney tissues will be examined for location of administered stem cells and vascular integration.

Example 7

Examine the *In Vitro* Transdifferentiation of MSC into Renal Tubular and Endothelial Cells

Spontaneous transdifferentiation of MSC generally does not occur. Treatment of MSC cultures with specific factors results in their transdifferentiation into adipocytes, osteocytes, chondrocytes and other cell types. Differentiation factors will be identified that result in transdifferentiation of MSC into tubular cells. The kidney is of mesodermal origin and during embryonal nephrogenesis ureteric bud cells induce a mesenchymal-epithelial transdifferentiation in the metanephric mesenchyme. This process is influenced by several growth factors (HGF, EGF, LIF, TGF alpha, FGF2) that exhibit redundancy and is critical to overall nephrogenesis, since failure of induction of the metanephric mesenchyme results in its apoptosis, and since the mesenchyme, on the other hand, induces the ureteric bud to undergo

branching morphogenesis which results in collecting duct formation. MSCs will be examined to determine the ability of the MSCs to transdifferentiate into tubular epithelial cells.

Cell culture systems will be utilized for MSC culture, including collagen (I or IV) culture; 3-D culture, co-culture with embryonic somites. The capacity of various differentiation and growth factors to induce the transdifferentiation of MSC into renal progenitor cells will be examined. Pax-2 will be used as an initial marker of tubular cell induction, since it is a kidney specific transcription factor that is expressed in the embryonic kidney, and that, importantly, is re-expressed in proximal tubular cells that are injured in ARF.

The *in vitro* system for induction of MSC into tubular cells will then be used for further analysis of molecular mediator signals and their utility for pre-differentiation of MSC that may be subsequently tested in ARF treatment protocols.

Example 8

Analyze *In Vivo* Transdifferentiation and Integration of Intrarenally Injected MSC (Subcapsular, Cortical Interstitium) and HSC in Intact and ARF Kidneys in Mice

HSC and MSC from eGFP transgenic mice will be injected (subcapsular or in midcortex) into normal and ARF kidneys of wild type mice. Their potential transdifferentiation and integration into tubular and vascular structures will be analyzed histologically, using appropriate differentiation markers as above. The data from these *in vivo* studies will determine whether MSC and/or HSC are able to transdifferentiate *in vivo* into specific renal cell types and the location of the injected cells. The influence of preexisting injury due to ARF on these processes will be assessed.

Example 9

Analyze the Effect HSC on Kidney Allograft Function

The effect of HSC, MSC and/or stem cell mobilization treatment on short- and long- term kidney allograft function will be analyzed. Kidney

transplantation will be performed using a two-step rat model. The donor will be a Fisher 344, male rat, transgenic for human placental alkaline phosphatase. The recipient will be a compatible Fisher 344 female wild type rat or an incompatible Lewis female wild type rat. Studies will be performed as described above using the kidney allograft rat model.

Although the invention herein has been described in connection with preferred embodiments thereof, it will be appreciated by those skilled in the art that addition, modifications, substitutions, and deletions not specifically described may be made without departing from the spirit and scope of the invention as defined in the appended claims, and all embodiments that come within the meaning of the claims, either literally or by equivalence, are intended to be embraced therein.

CLAIMS

1. A method of treating kidney dysfunction comprising delivering a therapeutic amount of non-transformed adult stem cells to a patient in need thereof.

2. The method of claim 1 wherein said adult stem cells comprise hematopoietic stem cells.

3. The method of claim 1 wherein said adult stem cells comprise mesenchymal stromal stem cells.

4. The method of claim 1 wherein said adult stem cells comprise non-hematopoietic pluripotent stem cells.

5. The method of claim 4 wherein said non-hematopoietic pluripotent stem cells comprise kidney derived pluripotent stem cells.

6. A method of treating kidney dysfunction comprising delivering a therapeutic amount of a stimulant of adult stem cell mobilization to a patient in need thereof;

wherein the stimulant mobilizes adult stem cells to the kidney.